**Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**IB Practical 4: Separation of Plant Pigments Using Chromatography**

Purpose: To identify plant pigments by separation and isolation of the pigments using **paper chromatography**.

Pre-Lab Questions: Please read the lab and answer the following questions based on the information provided.

1) What is photosynthesis?

2) What are the main plant pigments? What are the functions of these pigment?

3) What are accessory pigments? What are the functions of these pigments?

4) What is chromatography? How do paper chromatography and thin layer chromatography differ?

5) What is the Rf value?

6) How is the Rf value calculated?

7) What does the Rf value allow us to determine?

Background - Photosynthesis:

Plants are primary producers (along with some bacteria and algae) and produce their own food by using the sun’s energy to transform carbon dioxide and water into glucose. In this process of **photosynthesis**, plants convert the sun’s energy into chemical energy that is stored in the bonds of the glucose molecule. This energy fuels the metabolic processes of cells and is essential for life on earth. Glucose is a simple carbohydrate that provides immediate fuel to cells but it is also a building block for more complex carbohydrates stored by living organisms for future use.

For photosynthesis to transform light energy from the sun into chemical energy (bond energy) in plants, the pigment molecules absorb light to power the chemical reactions. **Plant pigments** are macromolecules produced by the plant, and these pigments absorb specified wavelengths of visible light to provide the energy required for photosynthesis. (see diagram below). Chlorophyll is necessary for photosynthesis, but **accessory pigments** such as carotenoids (carotene and xanthophyll) collect and transfer energy to chlorophyll. Although pigments absorb light, the wavelengths of light that are not absorbed by the plant pigments are reflected back to the eye. The reflected wavelengths are the colors we see in observing the plant. (Example: green pigments reflect green light) Plants contain different pigments, and some of the pigments observed include:

♣ chlorophylls a & b (greens)

♣ carotene (orange)

♣ xanthophyll (yellow)



Background - Paper Chromatography:

The process of **chromatography** separates molecules because of the different solubilities of the molecules in a selected solvent. In **paper chromatography**, paper marked with an unknown, such as plant extract, is placed in a developing chamber with a specified solvent. In **thin layer chromatography**, the solid phase is a glass slide with a material adhered to the surface (see related video). The solvent carries the dissolved pigments as it moves up the paper. The pigments are carried at different rates because they are not equally soluble. A pigment that is the most soluble will travel the greatest distance and a pigment that is less soluble will move a shorter distance.



The distance the pigment travels is unique for that pigment in set conditions and is used to identify the pigment. The ratio is the **Rf (retention factor) value**. Standards are available for comparison

Rf equation:

equation

Preparation of the Sample:

A. Take a strip of chromatography paper approximately 18 cm long. Cut it to fit the tube without touching the sides.Cut one end into a triangle. With a pencil lightly make a line 2 cm from the pointed end of the paper. 

B. Bend the strip of paper at the blunt end so it hangs over slightly. Adjust the length of the paper so that when it is inserted into the test tube, it will touch the bottom without curling.

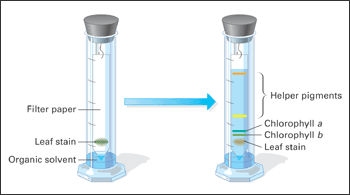


**Experiment part 2 -  Separating pigments using Chromatography**

Note: Only touch the edges of the chromatography paper.

STEP 1. PUT A LEAF INTO BOILING WATER FOR 3 MINUNTES, LET IT COOL, MASH IT UP WITH YOU PESTLE AND MORTAR, THIS IS YOUR CHLOROPHYLL MIXTURE

1. Draw a pencil line across the paper, 2.5cm from the bottom end. This is the base line
2. Put the "special solvent" (petroleum ether 10% & propanone 90%) into the tube up to 1.5cm depth.
3. Replace the parafilm and allow the air in the tube to become saturated with solvent (about 10 mins).
4. Take your strip and put it on two glass rods (or pencils) so that the loading point on your base line is not touching the bench.
5. Use a fine capillary tube or small metal tool to put a tiny spot of chlorophyll mixture onto the line on the paper, and allow to dry.
6. Add 10 more drops of pigment to the loading point.
7. Put the paper back into the tube, avoiding letting it touch the edges of the tube, replace the parafilm
8. Wait for the solvent to rise up the paper to 20cm from the bung. (keep looking)
9. Remove the paper and mark the solvent front (where the solvent migrated to) with a pencil line.
10. Hang up to dry (in a dark place if possible.)
11. When dry, mark the centre of each individual pigment spot on the paper and record its colour,



Analysis and Calculation of Rf values:

****1. On the Student Data Sheet, color the diagram to illustrate the color bands on the chromatogram. Label the band that traveled the greatest distance 1, the next 2, the next 3. Continue until all bands are labeled.

2. Describe the color of each band in Data Table.

3. Measure the distance from the first pencil line to the solvent front. Record this value in Data Table for each pigment.

4. Now measure the distance from the first pencil line to the average peak of each color band.

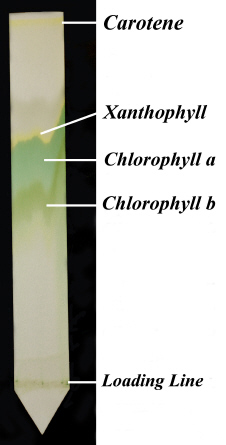
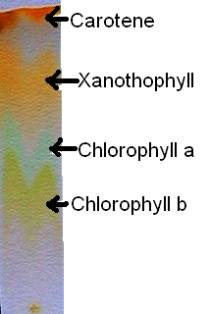
5. Record these values in Data Table. (Depending on the results, groups may have differing numbers of pigments.)

6. Calculate the Rf values for each pigment and record the values in Data Table using the following formula Rf = distance pigment travels/distance the solvent front travels

SOLVENT FRONT: 56mm

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| band | Band Color | Distance (mm) | Rf (Calculated) | Pigment |
| 1 | Yellow/green | 16 |  |  |
| 2 | Blue/green | 22 |  |  |
| 3 | yellow | 37 |  |  |
| 4 | Yellow/orange | 54 |  |  |

7. Use the examples below to determine the Rf of other samples and compare to your results to determine the name of each pigment and record the name in Data Table.

Conclusion Questions:

1) Explain why the four pigments moved at different rates through the chromatography paper.

2) Would any leaf from any plant have the pigments that are present in spinach or kale? How would this affect the chromatography with these different leaves?

3) A procedure known as thin layer chromatography would give even better results than chromatography paper. Research and explain the difference between thin layer chromatography and paper chromatography.